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INTERNATIONAL PRELIMINARY EXAMINATION REPORT  
(PCT Article 36 and Rule 70)

REC'D 21 MAY 2004

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Applicant's or agent's file reference 4 -32331A	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP 03/00803	International filing date (day/month/year) 27.01.2003	Priority date (day/month/year) 28.01.2002
International Patent Classification (IPC) or both national classification and IPC A61K47/48, A61K47/48		
Applicant NOVARTIS AG et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
  
2. This REPORT consists of a total of 7 sheets, including this cover sheet.  
 This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).  
These annexes consist of a total of 1 sheets.
  
3. This report contains indications relating to the following items:  

I	<input checked="" type="checkbox"/>	Basis of the opinion
II	<input type="checkbox"/>	Priority
III	<input checked="" type="checkbox"/>	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	<input type="checkbox"/>	Lack of unity of invention
V	<input checked="" type="checkbox"/>	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	<input type="checkbox"/>	Certain documents cited
VII	<input type="checkbox"/>	Certain defects in the international application
VIII	<input type="checkbox"/>	Certain observations on the international application

Date of submission of the demand 30.07.2003	Date of completion of this report 18.05.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Fausti, S Telephone No. +49 89 2399-7389



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/EP 03/00803

**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, Pages**

1-26 as originally filed

**Claims, Numbers**

8-16 as originally filed  
1-7 filed with telefax on 06.04.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.:
- the drawings, sheets:

5.  This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/EP 03/00803

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:
  - the entire international application,
  - claims Nos. 14-15 with respect to Industrial Applicability
    - because:
  - the said international application, or the said claims Nos. 14-15 relate to the following subject matter which does not require an international preliminary examination (specify):  
**see separate sheet**
  - the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
  - the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
  - no international search report has been established for the said claims Nos.
2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
  - the written form has not been furnished or does not comply with the Standard.
  - the computer readable form has not been furnished or does not comply with the Standard.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes:	Claims	1-16
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-16
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-13,16
	No:	Claims	-

**2. Citations and explanations**

**see separate sheet**

**Re Item III**

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

- N. Claims 14 and 15 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**DOCUMENTS.**

1. Reference is made to the following documents:

- D1: Garcia-Echeverria C. et Al., Bioorganic & Medicinal Chemistry Letters (2001) vol. 11, no. 11, pages 1363-1366;  
D2: Frackenpohl J. et Al., Chembiochem (2001) vol. 2, no. 6, pages 445-455;  
D3: EP 0560730 A;  
D4: Seebach D. et Al., Helvetica Chimica Acta (2001) vol. 84, no. 2, pages 271-279.  
D5: Wei-Chiang S. et Al., J. Biol.Chem. (1985) vol. 260, no. 20, pages 10905-10908 (added by IPEA);  
D6: Wei-Chiang S. et Al., Proc.Nat.Acad.Sci. USA (1978) vol. 75, no. 4, pages 1872-1876 (added by IPEA).

- 1.1 D1 discloses Antennapedia derived peptides as transport agents for the intracellular delivery of covalently linked molecular moieties (see abstract and page 1363, left-hand column, lines 1-7). In particular, D1 discloses conjugates of these peptides with fluorescein or cargo peptides, which are transported across the cell membrane (see table 1 and figures 1 and 2).  
1.1<sup>a</sup> D1 further disclose the synthetic approach for attaching the fluorescein moiety to the peptide N-terminus by means of the reaction between fluorescein

isothiocyanate and the immobilized peptide (see paragraph joining pages 1363 and 1364).

- 1.2 D2 and D4 disclose peptide analogues comprising 4-6  $\beta$ -Homolysine residues (see: D2, compounds 3, 4 and 6 on page 447; D4, compounds 2 and 3 on page 272; D2, compound 4 of figure 1).
- 1.2<sup>a</sup> In addition, D2 teaches that peptide analogues comprising  $\beta$ -amino acids have a high resistance towards proteolytic enzymes and therefore they are promising candidates as peptidomimetic moieties for the manufacture of pharmaceutical compounds (see abstract and page 453, left-hand column, second paragraph).
- 1.3 D3 discloses peptide derivatives comprising one  $\beta$ -amino acid residue having a  $\beta$ -Homolysine structure, for the treatment of thrombosis (see abstract and claim 1).
- 1.5 D5 and D6 disclose poly(Lysine) conjugates for the intracellular delivery of proteins or cytotoxic agents (see abstracts). In particular, D6 discloses conjugates wherein the poly(Lysine) transport agent binds to the cytotoxic agent through a cleavable linker (see abstract).

**2. NOVELTY (Art. 33(2) PCT).**

- 2.1 Claim 1 has been amended by introducing the feature of " $\beta$ -homolysine polymer". This amendment is supported by the application as originally filed (see for example page 1, lines 8-10 of the description) and therefore the requirements of Art. 34(2)(b) PCT are met.
- 2.2 In view of this feature, the subject-matter of independent claim 1 is novel over the available prior art.
- 2.2<sup>a</sup> In particular, the conjugate of claim 1 differs from the peptide analogues disclosed in D2 and D4 (see point 1.2 above) in the  $\beta$ -homolysine polymeric structure, which implies a consecutive sequence of  $\beta$ -Homolysine residues. In the peptide analogues of D2 and D4, there is no  $\beta$ -homolysine residue directly bound to another  $\beta$ -homolysine, but one or more amino acid residues are present between two successive  $\beta$ -homolysine units in the sequence. This corresponds to an interrupted (non-consecutive) sequence of  $\beta$ -homolysine residues, which is not to be considered a  $\beta$ -homolysine polymer.

- 2.2<sup>b</sup> In addition, the claimed conjugates differ from the peptide derivatives of D3 in that said  $\beta$ -homolysine polymer comprises at least four  $\beta$ -homolysine residues, rather than one of these residues only (see point 1.3 above).
- 2.2<sup>c</sup> Further, the  $\beta$ -amino acid structure of the Homolysine residues distinguishes the conjugate of claim 1 from the conjugates of D1, D5 and D6, which comprise consecutive sequences of the standard  $\alpha$ -amino acid lysine (e.g. poly-lysine polymers - see point 1.5 above). The presence of at least four monomeric units also distinguishes the claimed conjugate from the Antennapedia peptide conjugates disclosed in D1 because the Antennapedia peptide only comprises two consecutive lysines at the peptide C-terminus (i.e. Lys-57 and Lys-58) and two "isolated" lysines (i.e. Lys-46 and Lys-55), one of which is far removed from the others (see point 1.1 above).
- 2.4 As dependent claims 2-11 and 13, as well as claims 12 and 14-16, inherently or explicitly refer to the  $\beta$ -homolysine polymer feature, the subject-matter defined by these claims is novel for the same reasons.

3. INVENTIVE STEP (Art. 33(3) PCT).

- 3.1 Documents D5 and D6 are independently considered to represent the relevant state of the art because they disclose poly-lysine conjugates for delivering an active agent (cargo) into cells (across the cell membrane) (see point 1.5 above).
- 3.1<sup>a</sup> The subject-matter of claim 1 differs from these conjugates in that the transport agent comprises at least 4  $\beta$ -Homolysine residue, rather than lysine residues with the standard  $\alpha$ -amino acid structure.
- 3.2 The problem to be solved may therefore be regarded as the provision of alternative transport agents for delivering an active agent (cargo) into and/or across a biological barrier.
- 3.3 The solution proposed in the present application is to be considered as involving an inventive step because  $\beta$ -Homolysine polymers as defined in claim 1 have not been suggested for solving the problem posed, nor they can be considered an obvious alternative to the poly-lysines of D5 and D6.
- 3.3<sup>a</sup> Despite the  $\beta$ -Homolysine residue is described in D2 as being useful for the preparation of pharmaceutical peptide analogues, the behaviour of  $\beta$ -Homolysine polymers at/on the cell membrane (i.e. its biological barrier penetration property)

could not have been expected. In particular, peptide analogues comprising  $\beta$ -amino acids exhibits secondary structures, which differ from the ones of the corresponding  $\alpha$ -amino acid peptides. This fact might compromise (a priori) the barrier penetration property of  $\beta$ -Homolysine polymers with respect to poly-lysines. For example, D2 indicates that there is no guarantee that  $\beta$ -amino acid peptide analogues of medical interests will have good bioavailability and refers to the fact that absorption barriers may compromise the therapeutic potential of these analogues (see page 453, left-hand column, second paragraph).

- 3.4 The subject-matter of claims 1-16 is therefore to be considered as involving an inventive step because all these claims concern the use of  $\beta$ -Homolysine polymers as transport agents for delivering active agents into and/or across biological barriers.

**INDUSTRIAL APPLICABILITY (Art. 33(4) PCT).**

- IA.1 For the assessment of the present claims 14 and 15 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.
- IA.2 Claims 1-13 and 16 relates to pharmaceutical compounds (i.e. the conjugates of claims 1-11 and 13), methods for their preparation (claim 16), and pharmaceutical compositions thereof (claim 12). Said compounds, methods and compositions can be made or applied in the pharmaceutical industry, hence they are to be considered industrially applicable according to Article 33(4) PCT.

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CLAIMS

1. A conjugate that comprises a) at least one compound (CARGO) to be delivered into or across a biological barrier; b) a delivery-enhancing transporter (SHUTTLE) comprising at least 4 β-homolysine residues; c) optionally a linker (LINKER) between the components a) and b); and d) optionally a labelling unit (A); or a salt thereof.

2. A conjugate according to claim 1 having a structure selected from the group of structures (I) to (V),

A-SHUTTLE-CARGO-(CO)-Y (I),  
A-CARGO-SHUTTLE-(CO)-Y (II),  
SHUTTLE-LINKER-CARGO (III), and  
SHUTTLE-LINKER-CARGO-(CO)-Y (IV),

wherein Y is OR or NR<sub>1</sub>R<sub>2</sub> and wherein R, R<sub>1</sub> and R<sub>2</sub> independently of each other represent hydrogen or alkyl; or a salt thereof.

3. A conjugate according to claim 1 or 2 that comprises a delivery-enhancing transporter comprising between 4 and 25 β-homolysine residues; or a salt thereof.

4. A conjugate according to claim 1 or 2 that comprises a delivery-enhancing transporter comprising between 5 and 10 β-homolysine residues; or a salt thereof.

5. A conjugate according to any one of claims 1 to 4 wherein A is selected from biotinyl, fluorescein-5-yl and fluorescein-5-yl-NH-C(S)-NH-CH<sub>2</sub>-D<sub>r</sub>-E<sub>u</sub>-G<sub>p</sub>-CH<sub>2</sub>-C(O)-, wherein D, E and G are independently of each other selected from CH<sub>2</sub>, O or NH, under the proviso that not two heteroatoms are bonded to each other, and p, r and u are independently of each other an integer between 0 and 10; or a salt thereof.

6. A conjugate according to any one of claims 1 to 5 wherein the CARGO is a biomolecule selected from the group consisting of oligonucleotides, peptides and proteins; or a salt thereof.

7. A conjugate according to any one of claims 1 to 5 wherein the CARGO is an antibody; or a salt thereof.